

THE GENUS SHIGELLA

(DYSENTERY BACILLI AND ALLIED SPECIES)

ERWIN NETER

*Department of Bacteriology and Immunology, University of Buffalo, School of Medicine and
Laboratories of the Children's Hospital, Buffalo, New York*

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In 1898, Shiga first proved that dysentery of man may be caused by a bacillus, and thereby established the etiological distinction between bacillary and amebic dysentery. During a severe outbreak of dysentery in Japan, he isolated a bacillus, now called *Shigella dysenteriae* (Shiga), that was specifically agglutinated by the sera of patients with dysentery. In the two decades following this discovery, other bacilli were recovered from patients with epidemic or endemic dysentery and shown to be the causative agents of this disease. They differed, however, in certain respects from *S. dysenteriae* and are classified today as *S. paradysenteriae*, *S. sonnei*, and *S. schmitzii*. These four species have certain essential features in common and are considered, therefore, as members of one group: the genus *Shigella*. They are, furthermore, recognized as the cause of bacillary dysentery of man (true dysentery bacilli).

Other microorganisms which share certain morphological, cultural, biochemical, and antigenic characters with the true dysentery bacilli, and are, therefore, justifiably included in the genus *Shigella*, are not associated with epidemic or endemic bacillary dysentery of man. Although pathogenicity or lack of it is of prime importance to the medical bacteriologist, this characteristic alone is not a sound basis for the classification of bacteria. Unfortunately, the members of this genus which lack a relation to bacillary dysentery and, therefore, are

often considered to be non-pathogenic, have not received the same attention as have the true dysentery bacilli. Mention should be made, however, that some members of the genus which are not associated with dysentery, nevertheless, may be pathogenic for human beings and animals and cause diseases other than dysentery. Moreover, from the viewpoint of systematic bacteriology these members are as important as the true dysentery bacilli. Therefore, a review of the genus *Shigella* has to deal not only with those members that cause epidemic or endemic bacillary dysentery, but also with those species that are not specifically associated with this disease, that is, the allied species of Andrewes (7). The attempt is made here to present the pertinent data of the genus as a whole and to discuss certain features of the different species belonging to it.

THE GENUS SHIGELLA AS A WHOLE

It is rather difficult, at the present time, to give a definition of the genus *Shigella* which is sufficiently distinctive to differentiate it from others and yet broad enough to include all of its members. Moreover, it is safe to assume that some species now included in this genus may be eliminated and that others may be added in the future.

The members of the genus *Shigella* are commonly regarded as gram-negative, non-acid-fast bacilli, that have no spores and no capsules. The morphology of the various members offers no means of differentiation either from each other or from other non-motile, non-encapsulated, gram-negative bacilli of the tribe *Salmonelleae*. It is generally agreed that, with the possible exception of *S. sp.* (Newcastle type), whose taxonomic position is not as yet definitely established, the species of *Shigella* are non-motile and do not possess flagella.

In regard to the cultural characters, it may be pointed out that the majority of the members grow well on the usual culture media (including Endo agar) and are easily maintained thereon over long periods of time. They are aerobic, and most of them facultative anaerobic bacilli. However, it is important to note that certain species now included in the genus *Shigella* differ in their cultural characters: *S. septicaemiae*, for instance, thrives only in the presence of oxygen and fails to grow on Endo agar. These and other exceptions are generally overlooked in presentations of the genus as a whole.

Like many other bacteria, the members of the genus *Shigella*, too, produce different forms of colonies on agar plates. Unfortunately, dissociation has not always been adequately considered even in recent literature, and many contradictory statements can be found in regard to reversion among the different cultural phases, the sequence of phase transformation, and association of these phases with other characters of the microbes such as biochemical attributes, antigenic structure, and virulence. Arkwright (9) has described in detail the S and R forms of *S. dysenteriae* (Shiga). The former colonies are smooth, round, and domed and the latter flattened with irregular margins and surfaces. The smooth colonies emulsify without auto-agglutination in physiological salt solution, whereas the rough colonies show clumping. In addition to these smooth and rough colonies, intermediate colonies (Rs, RS, rS forms) are fre-

quently observed. The mucoid phase (M form), that occurs in many bacterial species, has been encountered also in members of the genus, for instance, in *S. equirulis*. Edwards (56) deserves credit for this important observation. Minute forms (dwarf or D colonies, G colonies¹ of Hadley (79) and G-like forms, *Zwergkolonien*) of certain bacterial species have been repeatedly reported both by European and American bacteriologists. Such forms have also been described among members of the genus *Shigella*, particularly, *S. dysenteriae*, *S. sonnei*, and *S. equirulis*. Dissociation and the various phases of bacterial growth among members of this genus, so far as they occur, will be considered with the description of the different species. This seems preferable to a general discussion, because dissociation in certain species of the genus has not as yet been studied adequately; and findings in one species do not necessarily hold true in others.

With respect to their biochemical activities, the members of the genus *Shigella* have but few reactions in common. Those members which are associated with epidemic or endemic bacillary dysentery of man (with the exception of *S. sp.* (Newcastle type)) produce acid but no gas from glucose, cause acid formation in litmus milk, reduce nitrates to nitrites,² form NH_3 , are Voges-Proskauer-negative, fail to form H_2S , do not grow in Koser's citrate medium, and do not liquefy gelatin (181). Some microorganisms now included in the genus, however, differ in their characters from the above; for instance, *S. sp.* (Newcastle type) produces acid and gas from glucose; *S. septicaemiae* liquefies gelatin, and so forth. Furthermore, it should be pointed out that some of the members of this genus have not been studied adequately in the past. For these reasons, any attempt to present the basic and common biochemical characters of the genus *Shigella* is beset with the greatest difficulties. Tables 1, 3, 4, 7, and 8 give the more important biochemical characteristics of the various members; because, at the present time, this seems more profitable than any such treatment of the genus as a whole.

Attention may be called to the appearance of variants having increased biochemical activity. The Sonne dysentery bacillus serves perhaps as the best example. On agar plates papillae or daughter colonies frequently appear which consist of raised, smooth, entire, rounded outgrowths on the surface. Characteristically, these daughter colonies, in contrast to the parent colonies, ferment lactose. The appearance of these papillae upon prolonged incubation seems to be responsible for the late fermentation of lactose rather than a slow utilization of this carbohydrate.

During the last few years our knowledge of the growth requirements of many microorganisms has increased considerably, although many questions remain unanswered. It is interesting to mention that important information on this subject has resulted from investigations on the mode of action of the sulfonamides. Until recently, little was known in regard to the more specific growth require-

¹ In this review, the term G colony is not restricted to those composed of filtrable elements alone.

² Some authors (149) differ on the question of reduction of nitrates.

ments of the various species of the genus *Shigella*. The observations of Koser and his associates (102, 50), therefore, are of particular interest. These authors found that many Flexner and Sonne dysentery bacilli either failed to grow or grew only poorly in a basic medium consisting of 15 amino acids, glucose and inorganic salts. Addition of nicotinamide or nicotinic acid exerted a marked growth-promoting effect. These substances, therefore, may be considered as essential growth factors for these strains. In passing, it may be mentioned that the stimulation of respiration due to nicotinamide is inhibited by sulfapyridine (51). It will be of interest to see whether the other species of the genus have similar or different growth requirements.

Whether or not the genus *Shigella* is characterized by an antigenic component which is common to all its members and differentiates it from other genera (genus-specific antigen) can not be definitely answered at the present time, particularly, since the antigenic structure of some of the members of the group has not as yet been thoroughly investigated. It is highly desirable to know whether or not such a genus-specific antigen exists; its demonstration would aid greatly in the identification of unknown strains and in the classification of species whose taxonomic position has not been firmly established. A discussion of the antigenic structure of the more important members of the genus will be presented below.

A primary subdivision of the genus *Shigella* is best obtained by classifying its members according to their action upon mannitol and lactose. Thus, four main groups are obtained, whose more important members are presented in table 1. As outlined in the introduction, the genus is further subdivided into true dysentery bacilli and allied species according to the relation of the respective species to epidemic and endemic bacillary dysentery of man. Fortunately, this primary subdivision of the genus according to the biochemical characters of its members is compatible with the equally important classification based upon the antigenic structure.

The members of the genus which, at the present time, are recognized as the cause of epidemic and endemic dysentery in man, are: *S. dysenteriae* (Shiga); *S. schmitzii*; *S. sp.* (Newcastle type); *S. paradyserteriae*; and *S. sonnei*. Unfortunately, these true dysentery bacilli are referred to in the literature under a variety of names. Table 2 presents a comparative classification and the respective terminology of different authors.

A few facts pertinent to the bacteriological and immunological aspects of bacillary dysentery may be mentioned. Bacillary dysentery of man, characterized by a more or less severe inflammation of the large intestine, may be epidemic, endemic, or sporadic. It may occur spontaneously also in monkeys and dogs. Sometimes, acute bacillary dysentery of man is not terminated by recovery, but continues as a subacute or chronic colitis. However, it has not as yet been conclusively shown that dysentery bacilli or other intestinal microorganisms play a significant pathogenic role in this form of colitis.

The bacteriological diagnosis of bacillary dysentery is based upon the isolation of dysentery bacilli from the feces. This may be carried out successfully

in a relatively high percentage of cases, provided that the bacteriological examination is done in the first few days of the disease and appropriate methods are

TABLE 1
A primary classification of the members of the genus Shigella

	GROUP I LACTOSE-NEGATIVE MANNITOL-NEGATIVE	GROUP II LACTOSE-NEGATIVE MANNITOL-POSITIVE	GROUP III LACTOSE-POSITIVE MANNITOL-NEGATIVE	GROUP IV LACTOSE-POSITIVE MANNITOL-POSITIVE
True dysentery bacilli	<i>S. dysenteriae</i> <i>S. schmitzii</i>	<i>S. paradysenteriae</i>		<i>S. sonnei</i>
Allied species	<i>S. ambigua</i> <i>S. septicaemiae</i> <i>S. minutissima</i>	<i>S. alkalescens</i> <i>S. gallinarum</i> <i>S. pfaffii</i> (?) <i>S. rettgeri</i> (?)	<i>S. gintottensis</i> <i>S. bienstockii</i> (?) <i>S. oxygenes</i> (?)	<i>S. equirulis</i> <i>S. ceylonensis</i> B <i>S. madampensis</i>

TABLE 2
*Comparative classification of the true dysentery bacilli according to different authors**

BERGEY, ET AL.	ANDREWES AND INMAN	KRUSE	LENTZ AND FRIGGE	SONNE	AOKI
<i>Shigella dysenteriae</i> (Shiga) Castellani and Chalmers	<i>Bacillus</i> <i>Shiga</i>	True dysentery bacillus	Shiga-Kruse bacillus	Shiga-Kruse bacillus	Group VIII
<i>Shigella ambigua</i> (Andrewes) Weldin	<i>Bacillus</i> <i>ambiguus</i>	Pseudo-dysentery bacilli I and J	Schmitz bacillus		
<i>Shigella paradysenteriae</i> (Collins) Weldin†	<i>Bacillus</i> <i>Flexner</i> — Y	Pseudo-dysentery bacilli			
	Y V (?)	B	Flexner bacillus	Group II	
	VZ	A	Y bacillus (Hiss-Russel)	Group I	Group I
	Y	D			Group X
	Z	H			Groups II, III, IV, V (?)
	WX X	C F			
	W (?)	G	Strong bacillus	Group II	
<i>Shigella sonnei</i> (Levine) Weldin	<i>Bacillus</i> <i>dispar</i>	Pseudo-dysentery bacillus E	Kruse-Sonne bacillus	Group III	

* Compiled after Lentz and Frigge (109).

† See also Table 6.

employed. In the majority of cases of bacillary dysentery the causative microorganisms remain localized in the intestinal tract and in the regional lymph glands. In some cases the dysentery bacilli may be found in the feces in almost

pure culture; in many cases, however, they represent only a small percentage of all the colonies cultivated, or, they may not be isolated at all. In the latter instance, the possibility should be considered that bacteriophage may be present in the feces. The demonstration of bacteriophage may aid in the diagnosis of bacillary dysentery (61, 190).

Invasion of the blood stream by dysentery bacilli is rare, although this may occur (152, 90, 90a, and others). Occasionally, the true dysentery bacilli such as the Shiga, Flexner, and Sonne bacilli may invade other organ systems and cause infections, for example, of the urinary tract (152, 137, 138, 142).

The technical procedures used for the isolation of dysentery bacilli from the feces will be mentioned but very briefly. It is important to examine the feces as soon as they are obtained; particles containing pus and blood are suspended in physiological saline solution or in infusion broth. When immediate examination of the specimen is not possible, the material can be preserved best in a buffered saline solution (pH 8.5) containing 1% sodium citrate and 0.5% sodium desoxycholate (16). This procedure deserves more widespread use in hospital laboratories. It is advisable to seed several agar plates with the suspension and to use a number of differential culture media such as Endo agar, MacConkey agar, eosin-methylene-blue-lactose agar, desoxycholate-citrate agar, lithium chloride-Endo agar, or others. Bismuth sulfite agar, that is so successfully used for the isolation of typhoid bacilli, is not suitable for the recovery of dysentery bacilli.* Recent studies indicate that desoxycholate-citrate agar allows heavier seeding than MacConkey agar and gives superior results (122, 86, 93a, and others). Inoculation of the plates is carried out in this laboratory as follows: a small area (approximately one-third) of each plate is first seeded; then, the loop is sterilized and cooled, and material from the first inoculation of the plate is taken up and streaked on another part of the same plate (second third); material from the second inoculation is then taken up for seeding of the remaining part of the plate. Thus, isolated colonies are usually obtained. After incubation for 18 to 24 hours at 37 C non-lactose-fermenting colonies are sub-cultured. Fishing of lactose-negative colonies after further incubation may increase the percentage of successful isolations (122). These colonies are then studied in regard to morphology, motility, cultural characters, biochemical activities, and antigenic structure. In this laboratory, fermentation tubes are used with glucose, mannitol, and dulcitol. Since autoclaving at 15 pounds causes a partial breakdown of some test substances, thus leading to false reactions, it is advisable to sterilize broths containing lactose, maltose, sucrose, rhamnose, and xylose by filtration through Seitz filters. Autoclaving may not produce a noticeable change in pH; however, organisms which do not ferment the original substances may act upon the breakdown products with the formation of acid, or acid and gas. Such reactions must be carefully avoided.

* Very recently, Wilson and Blair (192a) described a new culture medium selective for Flexner dysentery bacilli. On this tellurite-iron-rosolic acid agar Flexner dysentery bacilli grow profusely, whereas *E. coli* and *A. aerogenes* are largely inhibited. Thomas and Hulme (180a) have confirmed the usefulness of this medium.

For diagnostic agglutination tests sera of immunized rabbits are preferable. When antisera of horses are employed, it should be kept in mind that the titer of normal agglutinins is relatively high and certainly above that of rabbit and human sera.

The serological diagnosis of bacillary dysentery (Widal test) presents greater difficulty by far than does that of typhoid fever; this is particularly true in cases with infections due to members of the Flexner group. Whenever the serological diagnosis of infections due to species of *Shigella* is carried out, it is essential (a) to use standardized suspensions of known agglutinability; (b) to employ a standardized procedure (temperature, time of incubation, etc.); (c) to know the titer of normal agglutinins for the respective suspensions; and (d) to employ proper positive and negative controls. For the diagnosis of mixed infections with two or more species of *Shigella*, the Castellani test may be used successfully. The uncritical employment of the Widal test for the diagnosis of bacillary dysentery has, unfortunately, done much to discredit this method; some authors, for example, have postulated a certain titer of agglutinins for *all* types of dysentery bacilli as diagnostic of previous or present infection without due consideration of the points just outlined.

The following therapeutic sera have been used successfully in the treatment of bacillary dysentery of man and of experimental infections of animals: (a) Immune sera containing antibodies directed against the exotoxin of Shiga's bacillus (anti-exotoxin); and (b) immune sera containing antibodies directed against the dysentery bacilli themselves or their endotoxins (immune-opsonins or tropins, bactericidal antibodies and anti-endotoxins) (65, 19a). Human convalescent serum has also been recommended (63).

A second class of specific agents for the treatment of such infections is bacteriophage. There is a considerable literature on the subject, but it is not yet possible to make a final statement as to the efficacy of this treatment. Certain authors believe that the presence of highly effective bacteriophage may terminate the disease and that its therapeutic application is of definite value (91, 182, 160, and others). Others, however, feel that the oral administration of bacteriophage does not alter the clinical course of acute bacillary dysentery (155).

Much more promising than treatment with either bacteriophage or anti-bacterial immune serum are chemotherapeutic compounds of the sulfonamide type. Space does not permit a detailed discussion; a few data, however, may be given. Certain sulfamido compounds are definitely bacteriostatic toward dysentery bacilli. Moreover, Levaditi and Vaisman (110) demonstrated that sulfanilamide and related compounds prevent the effects of certain dysenteric endotoxins *in vivo*. However, the role of this 'anti-endotoxic' action of sulfonamides in the treatment of spontaneous *Shigella* infections of animals and man remains to be determined. Sulfathiazole and sulfaguanidine have proved to be efficacious in the treatment of experimental infections of animals and of bacillary dysentery of man (Marshall and associates (121), Weil and Gall (187), Lyon (115), Cooper and Keller (44, 45), and Libby (114)). Sulfaguanidine and similar compounds seem to be particularly suitable for the treatment of

this disease, since they inhibit the growth of members of the genus *Shigella*, are fairly soluble in water, and are only poorly absorbed from the intestinal tract. Thus, relatively high concentrations of the drugs can be obtained at the site of the infection.

In regard to specific prophylaxis of bacillary dysentery, active immunization has been attempted by means of (a) Shiga exotoxin-antitoxin mixtures; (b) Shiga toxoid; and (c) killed bacterial suspensions. With war raging in parts of the world where bacillary dysentery is endemic, the prevention of this disease by means of active immunization is of particular interest now and will be further discussed below.

GROUP I. THE LACTOSE-NEGATIVE, MANNITOL-NEGATIVE MEMBERS

1. *S. dysenteriae* (Shiga) Castellani and Chalmers is the type species of the genus *Shigella*. It is a gram-negative, non-motile bacillus; it has no capsule and forms no spores. It grows well on ordinary culture media; the optimal temperature for its cultivation is about 37 C. Upon incubation for 24 hours on plain agar or MacConkey agar colonies in the S phase are round, shiny, smooth, sharply defined, domed, and translucent. Colonies in the R phase may be either flat and thin with irregular outlines and rough surfaces, or they may be moderately thick, but flattened, with indented or slightly jagged margins. Generally, after 24 hours of incubation, the R forms of Shiga's bacillus are somewhat larger than the S forms. In addition to typically smooth and rough colonies, intermediate forms are frequently seen. R variants may develop from S strains spontaneously, particularly in old cultures, or under a variety of different conditions, for instance, in the presence of bacteriophage. Arkwright (9), whose investigations of the different cultural forms of the Shiga dysentery bacillus are of outstanding importance, found that changes of the culture phase are associated with changes of other attributes. The S forms give stable suspensions in physiological salt solution and cause uniform turbidity in broth cultures. In contrast, the R forms agglutinate spontaneously in physiological salt solution and form a deposit in broth cultures. Reason for this different behavior of S and R forms of *S. dysenteriae* is of interest. White (191) found that agglutination of the R form of Shiga's bacillus in physiological salt solution can be prevented, if the alcohol-soluble substances of the bacilli are previously removed. It seems desirable to investigate the nature of these alcohol-soluble substances. S and R forms differ also in their antigenic structure, as will be discussed below. In addition to these normal sized colonies, cultures of Shiga's bacillus may occasionally show very small colonies, the so-called G or G-like colonies. There cannot be any doubt about the existence of this colony form. On the other hand, it remains to be seen whether or not elements of these colonies are filtrable. Attempts to confirm the original observations (80) of a filtrable variant of *S. dysenteriae* have not been successful (79). This, however, should not be interpreted to indicate that filtrable forms of bacteria in general do not exist. This particular phase of the problem of bacterial dissociation will be discussed further in the section on *S. sonnei*.

The biochemical reactions of the Shiga bacillus are very characteristic. They are presented in table 3. As may be seen from table 3, *S. dysenteriae* differs in its biochemical reactions from the other members of the group. Its failure to form indole clearly differentiates the Shiga from the Schmitz bacillus, the other member of the group which may cause dysentery of man.

Antigenically, the R and S forms of the Shiga dysentery bacillus are distinct. This was shown by Arkwright (9) in 1921. Failure to correlate bacterial dissociation and accompanying changes in the antigenic structure of the Shiga bacillus accounts for many reports in the literature on so-called inagglutinable strains. Arkwright showed that rabbits immunized with pure phases of either R or S forms develop agglutinins against the homologous organism; these antisera give only very little cross-reaction with the heterologous organism.

TABLE 3

Biochemical reactions and antigenic structure of the lactose-negative, mannitol-negative group

	INDOLE	LITMUS MILK	GLUCOSE	LACTOSE	SUCROSE	MANNITOL	MALTOSE	RAMNOSE*	XYLOSE	DULCITOL	ANTIGENIC STRUCTURE
<i>Shigella dysenteriae</i> (Shiga) Castellani and Chalmers	—	A-Alk	A	—	—	—	—	—	—	—	Homogeneous
<i>Shigella schmitzii</i> , Hauduroy, et al.	+	A	A	—	—	—	— occas. A	A	—	—	Homogeneous
<i>Shigella ambigua</i> (Andrewes) Weldin	+	A-Alk	A	—	— occas. A	—	—	A	—	—	Incompletely studied
<i>Shigella septicaemiae</i> (Bergey, et al.) Bergey, et al.	+	A or —	A	—	—	—	—	—	—	—	Unknown
<i>Shigella minutissima</i> (Migula) Bergey, et al.	—	AC	A	—	—	—	—	—	—	—	Unknown

A = Acid reaction.

C = Coagulation.

Alk = Alkaline reaction.

+= Indole production.

— = No indole or acid production.

* Synonym for isodulcitol.

The S and R forms of all strains are agglutinated by the respective antiserum. It is now generally agreed that in its smooth phase this species is antigenically homogeneous. Arkwright noted, furthermore, that the S form is agglutinated in large clumps by specific antiserum, whereas the R form is agglutinated in small clumps which are readily shaken up into a turbid suspension. Absorption experiments give further evidence of the distinct antigenic structure of these two cultural phases. Absorption of a polyvalent serum (which agglutinates both R and S forms) with the S form eliminates the antibodies to the latter without reducing to any great extent the titer of the antibodies to the R form; and *vice versa*, the R form absorbs its own agglutinins from such a serum without markedly reducing the titer of the antibodies to the S form.

The chemical analysis of the various antigenic substances, which give the characteristic immunological pattern to bacterial species and their variants, has yielded interesting information in the past. Such investigations of Shiga's dysentery bacillus have been particularly fruitful at the hands of Morgan and his associates (129, 130). Similar studies on other species of the genus *Shigella* are highly desirable.

The antigen in smooth strains of the Shiga bacillus which gives them their species-specificity has been isolated and purified (129, 130, 150). This antigen consists of three main components: (a) a polysaccharide, (b) a polypeptide-like substance, and (c) a phospholipin. The latter seems to be of minor significance. The polysaccharide absorbs not only the agglutinins to the Shiga bacillus, but also the heterophile antibodies, which may be induced by the injection of the whole antigen-complex or of intact bacilli. This heterophile hapten of the Shiga bacillus was first demonstrated by Iijima (93), who found that rabbits injected with a suspension of Shiga's bacillus formed sheep-cell hemolysin of the Forssman type. According to the investigations of Meyer and Morgan (124, 125), the formation of these antibodies is induced not by two different substances, but by a single polysaccharide with two distinct reactive groups: one reacting with the agglutinin to the Shiga bacillus, the other with the sheep-cell hemolysin. This observation is of great general immunological interest. The polysaccharide is a typical hapten; alone, it does not induce the formation of antibodies *in vivo*. The polysaccharide-polypeptide mixture engenders antibodies against both components (150). It is interesting to note that antibodies against the polypeptide are not present in immune sera prepared by the injection of the organisms themselves. The significance of these antibodies in immunity remains to be determined. All strains of the Shiga dysentery bacillus do not contain the heterophile sheep-cell hapten. Shiga's bacillus may lose the sheep-cell hapten upon treatment with bacteriophage (28). The presence or absence of this heterogenetic sheep-cell hapten depends not only upon the individual strain, but also upon the composition of the culture medium (60). It is evident that any such changes in antigenic structure should be carefully correlated to possible changes of the culture phase of the organism. This, however, has not always been done in the past. In addition to the species-specific bacterial polysaccharide, the Shiga bacillus may contain other antigenic components, for instance, antigens in common with human blood cells (58, 59, 162). The significance of the Forssman and blood-group antigens in Shiga's bacillus and, particularly, their relationship to pathogenicity, remain to be determined.

The chemical structure of the antigen or antigens characteristic of the R phase of the Shiga dysentery bacillus is not known as yet.

Levine and Frisch (112) and Burnet (31 to 34) made the important observation that a relationship exists between the effectiveness or ineffectiveness of isolated strains of bacteriophage and the antigenic structure of the respective bacterial strain. Arkwright (10), in 1924, reported that from S forms of Shiga's

bacillus, which are resistant to bacteriophage, phage-susceptible variants may be obtained. It is interesting to note that these variants are always R forms.

A few years following the discovery of the Shiga dysentery bacillus, it was found that sterile culture filtrates were toxic for rabbits, causing diarrhea, paralysis of the extremities (due to lesions in the central nervous system) and death (43, 134, 158). The exotoxin of *S. dysenteriae* is often referred to as a neurotoxin, because it affects the nervous system. It is highly reactive in rabbits and horses and less so in mice, guinea-pigs, and rats. It may be obtained by filtration of broth cultures, an incubation for two weeks yielding smaller amounts of exotoxin than incubation for three to four weeks. The exotoxin may also be obtained from the bacterial cells themselves: for instance, by repeated freezing and thawing, by shaking, by chemical methods, and also by the action of bacteriophage. The latter procedure was used successfully by Kuhn (106). It is generally true that, so far as members of the enteric group of bacilli are concerned, strains in the S phase are more toxic than in the R phase. The observation of Thibault and Braunberger (178) who reported that smooth and rough colonies of a Shiga strain were equally toxic, thus requires confirmation.

During recent years, several attempts have been made to separate the exotoxin of the Shiga bacillus from the endotoxin. Olitsky and Kligler (146), Boivin and Mesrobian (20 to 22), and Haas (76) claim to have accomplished this separation. These authors report, furthermore, that the exotoxin affects the nervous system (neurotoxin), whereas the endotoxin causes lesions of the intestinal tract (enterotoxin). This opinion, however, has not been generally accepted (184).

Formaldehyde exerts a characteristic influence upon the exotoxin in reducing its toxicity without diminishing its antigenic properties to any great extent. The formaldehyde-treated exotoxin (toxoid) of Shiga's bacillus is not completely devoid of toxicity (83). The toxoid may be successfully used for the immunization of animals in order to obtain high-titered antisera for therapeutic purposes. The mortality rate of the animals injected with toxoid is markedly lower than that of the animals immunized with the untreated toxin. This toxoid possibly may be employed also for the active immunization of man and may prove valuable now, with war raging in areas where Shiga bacillus infections are endemic.

Cultures of the Shiga dysentery bacillus yield a filtrable substance of toxin-like nature which elicits the so-called Schwartzman phenomenon (167). An effective filtrate is obtained by growing a suitable strain on agar surface, washing the growth off with physiological salt solution and passing the fluid through a Berkefeld filter. When such a filtrate is injected into the skin of rabbits, followed 24 hours later by an intravenous injection of the same or certain other filtrates, a severe hemorrhagic-necrotic lesion appears at the site of the first injection. In spite of the fact that this phenomenon has been investigated extensively in animals, the role of the Schwartzman factor in the pathogenesis

of bacillary dysentery of man is by no means clear, especially since it is not even known whether this substance is produced under natural conditions of infection.

Brief mention may be made of the reported existence in Shiga bacilli of substances which induce allergic reactions in sensitive individuals (169). The nature of these allergens and their significance in bacillary dysentery of man remain to be determined.

2. *S. schmitzii* Hauduroy, *et al.* 3. *S. ambigua* (Andrewes) Weldin. In 1917, Schmitz (164) isolated from patients with clinical signs of dysentery a bacillus which showed certain similarities to Shiga's bacillus and yet revealed striking differences. A similar microorganism was studied by Andrewes (7) in 1918, who referred to it as *Bacillus ambiguus*. At the present time, it is impossible to decide whether all organisms identified as either *S. schmitzii* or *S. ambigua* are identical or differ in some as yet unknown properties. Not until these strains have been studied adequately, will it be possible to state whether these organisms form one single or two different species. It is true that Schmitz's bacillus has been recognized as a cause of bacillary dysentery (164, 163), and that Andrewes' bacillus was considered to bear no relationship to dysentery of man. In this connection it may be pointed out that the factors responsible for pathogenicity of certain strains and species are still very incompletely known. Furthermore, it has not been explained why one species (*e.g.*, *S. paradysenteriae*) may cause epidemic outbreaks of dysentery in man and a very closely related species (*e.g.*, *S. alkalescens*) does not. In the latest edition (1939) of Bergey's Manual of Determinative Bacteriology (19) and in A System of Bacteriology in Relation to Medicine (72) only one species is recognized. An extensive study of all features of these microorganisms is definitely needed, and it should be kept in mind that such closely related organisms as *S. paradysenteriae* and *S. alkalescens* are recognized today as two distinct species.

S. schmitzii resembles Shiga's bacillus, but it may readily be distinguished from the latter by its capacity to form indole and to produce acid from rhamnose (table 3). Antigenically, this species is homogeneous (161) and distinct from Shiga's bacillus. Antisera to the Schmitz bacillus obtained from rabbits agglutinate Shiga's bacillus only slightly or not at all. Likewise, antisera to Shiga's bacillus fail to agglutinate to high titer the bacillus of Schmitz. When cross-reactions are encountered, agglutinin-absorption tests allow a definite differentiation of the two organisms. Another important difference between the two species exists with respect to their toxicity, the Shiga bacillus producing a powerful exotoxin, while the other does not.

4. *S. septicaemiae* (Bergey, *et al.*) Bergey, *et al.* 5. *S. minutissima* (Migula) Bergey, *et al.* Both of these microorganisms were first described many years ago and have received but little attention recently. Only a few data are available. A thorough reinvestigation of these two species is definitely needed. Otherwise, the incomplete information will be carried from edition to edition of the manuals of bacteriology. Only when the characters of these organisms are known adequately, will it be possible to identify unknown strains, to study

the incidence and pathogenicity, and to reconsider the taxonomic position of these two species.

S. septicaemiae was isolated in 1904 by Riemer (156) from geese and referred to as "*Bacillus septicaemiae anserum exsudativae*." It is a small rod (0.1 by 0.3 to 1.0 μ). According to Riemer, it thrives only in the presence of oxygen and thus differs from other members of the group which are facultative anaerobes. Apparently, the growth requirements of this organism differ from those of the majority of the members of the genus *Shigella*, inasmuch as *S. septicaemiae* fails to grow on Endo agar. In a medium containing glucose it causes a change of the pH to the acid side. It fails to ferment lactose and mannitol (table 3), and may form H_2S and traces of indole. It liquefies gelatin.

S. minutissima was first isolated in 1896 by Kruse (104) from an abscess and described as "*Bacillus pyogenes minutissimus*." It is a small rod (0.5 by 1.0 μ). It produces acid from glucose, but not from mannitol and lactose (table 3). It causes acid and clot formation in litmus milk. It fails to produce indole and does not liquefy gelatin.

6. *S. sp.* (Newcastle type). In 1929, Clayton and Warren (41, 42) isolated from patients presenting symptoms of dysentery a microorganism which they considered to be the cause of the disease and which differed from previously described species. Since then, this species has been encountered also in the United States.

At the present time, this microorganism is regarded as a member of the genus *Shigella* (19). It must be pointed out, however, that this bacillus differs in certain characters from all other members of this genus. In the first place, it is considered to be somewhat motile. In the second place, it produces small amounts of gas from certain carbohydrates in peptone-water and even larger amounts when grown in Lemco-broth. Its taxonomic position, therefore, has to be reconsidered. The fact that this microorganism may cause a dysentery-like disease in man, is not sufficient reason to include it in the genus *Shigella*.

S. sp. (Newcastle type) produces acid and gas from glucose, maltose, and dulcitol; it fails to attack lactose, sucrose, and mannitol; it causes acid formation in litmus milk and does not produce indole. Strains similar to the Newcastle bacillus, but fermenting mannitol have been described (52).

Antigenically, this species is homogeneous, according to the investigations of Clayton and Warren (41, 42). It is interesting to note that the Newcastle bacillus bears no antigenic relationship to Shiga's bacillus, but gives cross-reactions with certain members of the Flexner group. It may be mentioned in this connection that patients with dysentery due to *S. sp.* (Newcastle type) may develop specific agglutinins. Normal human sera fail to agglutinate this organism.

GROUP II. THE LACTOSE-NEGATIVE, MANNITOL-POSITIVE MEMBERS

7. *S. paradysenteriae* (Collins) Weldin is often referred to as the Flexner group of dysentery bacilli. As a typical member of the genus *Shigella*, it is a non-motile, gram-negative bacillus. Its average size from mature agar

cultures is 0.5 by 1.0 to 1.5 μ . It grows readily on ordinary culture media, including Endo agar. On plain agar the colonies may have the typical features of S, R, or S R forms. The R forms may occur spontaneously, particularly in old cultures; or they may be obtained by growing S forms in broth containing homologous antiserum, bacteriophage, or certain disinfectants such as phenol or formaldehyde. Papillae are less frequently seen with members of the Flexner group than with Sonne's bacillus. On lactose-containing media these papil-

TABLE 4
Biochemical reactions and antigenic structure of the lactose-negative, mannitol-positive group

	INDOLE	LITMUS MILK	GLUCOSE	LACTOSE	SUCROSE	MANNITOL	MALTOSE	RAHAMNOSE	XYLOSE	DULCITOL	ANTIGENIC STRUCTURE
<i>Shigella paradysenteriae</i> (Collins) Weldon	+ occas. —	A-Alk	A	—	— or A	A	A or —	—*	—	—*	13 or more types
<i>Shigella alkalescens</i> (Andrewes) Weldon	+	A-Alk	A	—	— occas. A	A	A	A	A	A	2 types
<i>Shigella gallinarum</i> , (Klein) Weldon	—	— (Alk ?)	A	—	—	A	A	A	A	A	Homogeneous (O- antigen of Group D of genus <i>Salmonella</i>)
<i>Shigella pfaflii</i> (Hadley, et al.) Weldon	—	—	A	—	—	A	A	A	A	—	Incompletely studied
<i>Shigella rettgeri</i> (Hadley, et al.) Weldon	— (+ ?)	Alk	A	—	—	A	—	?	A	—	Incompletely studied

A = Acid reaction. + = Indole production.
Alk = Alkaline reaction. — = No indole or acid production.
* On prolonged incubation, acid may be produced by some strains.

TABLE 5
Old classification of the varieties of Shigella paradysenteriae according to sugar fermentations

VARIETY	GLUCOSE	MANNITOL	MALTOSE	SUCROSE
Flexner	A	A	A	—
Hiss	A	A	—	—
Strong	A	A	—	A

A = Acid production; — = no acid production.

lae remain lactose-negative (74). On media containing other carbohydrates such as maltose and sucrose, these daughter colonies may show increased fermenting power.

The more important biochemical reactions of the Flexner group are summarized in tables 4 and 5. All of its members produce acid without gas from glucose and mannitol. Differences in biochemical activity of various strains with respect to the fermentation of maltose and sucrose were used in the past

for the subdivision of the Flexner group into the following varieties: (a) Flexner variety; (b) Hiss-Y-Russell variety; (c) Strong variety (table 5). These fermentation reactions, however, are neither constant in all instances, nor does the resulting subdivision parallel the more important classification based upon the antigenic structure. Unfortunately, the biochemical classification of the Flexner group is still largely used in the clinical literature; it should be abandoned.

To the medical bacteriologist the differentiation of the Flexner group from other members of the genus *Shigella* is of great importance, particularly, because the epidemiological significance of this group differs greatly from that of closely related species. As may be seen from table 4, indole formation and action upon rhamnose, xylose, and dulcitol allow a preliminary differentiation of the Flexner group from other lactose-negative, mannitol-positive members of the genus *Shigella*. *S. paradysoenteriae* differs from *S. alkalescens* by its inability to produce acid from rhamnose, xylose, and dulcitol. It must be mentioned, however, that a few strains of the Flexner group produce acid from rhamnose after incubation for several days (144, and others). It has also been reported that an occasional strain of the Flexner group may ferment dulcitol on prolonged incubation. As will be mentioned below, members of the Flexner group can readily be differentiated from *S. alkalescens* by serological methods.

S. paradysoenteriae is comprised of antigenically different types. Investigators in different countries have studied the antigenic structure of various strains and proposed different classifications,—a fact which adds more difficulties to an already difficult subject. Table 6 presents the various antigenic types of *S. paradysoenteriae*, as designated by different authors.

Andrewes and Inman (8) have subdivided the Flexner group into five different types, referred to as *S. paradysoenteriae* V, W, X, Y, and Z. These authors believe that the various members of the group contain four different antigenic components (V, W, X, and Z) and that the respective type is determined by the predominance of one or another of these antigens. The presence of minor and varying amounts of the other antigenic components explains the well-known cross-reactions occurring between the different types. In *S. paradysoenteriae* Y the four antigenic components V, W, X, and Z are rather evenly distributed; it remains doubtful, however, whether this type also contains a fifth antigenic component (Y).

There can be no doubt that the Flexner group is comprised of more than the five original types of Andrewes and Inman (table 6). Sartorius and Reploh (161) have described four additional types, three of which correspond to types reported by Aoki and Murakami. Their data do not completely correspond to those of Lentz and Prigge (109) (see tables 2 and 6). The most significant contribution to this subject is that of Boyd (24 to 27), who made an extensive study of the antigenic structure of some four thousand strains belonging to the mannitol-fermenting group of dysentery bacilli. About 75% of *S. paradysoenteriae* strains are classified as belonging to the five Types V, W, X, Y, and Z of Andrewes and Inman. The remaining 25% of his strains are comprised of

about nine additional types. A study of variants of these organisms has led Boyd to a new conception of the antigenic structure of the members of this species, which differs essentially from that of Andrewes and Inman. Boyd gives experimental evidence that the members of the Flexner group may contain a group-specific (species-specific) antigen in addition to type-specific antigens. The group-specific antigen itself is comprised of about six components, some of which are common to various types of the Flexner group. On the other hand, the type-specific antigen characterizes the respective type only. Thus, according to Boyd, the cross-reactions encountered with different types of the Flexner group, are due to common group-specific antigens. At the present time, 13 (possibly 15) different types of *S. paradysenteriae* can be recog-

TABLE 6

Classifications of the antigenic types of Shigella paradysenteriae according to different authors

BOYD	ANDREWES AND INMAN	KRUSE	SARTORIUS AND REPLOH	AOKI AND MURAKAMI	SUGGESTED TYPE DESIGNATION
V	V	BC	BC	V	1
W	W	D	D	I	2
X	X	—	X	X	3
Z	Z	H	H	II	4
88	—	—	K(L?)	IX (?)	5
103	—	—	—	—	6
P119	—	—	—	—	7
....	Y	—	Y	—
....	VZ	A	A	—
170	—	—	—	—	8
P288	—	—	—	—	9
P274	—	—	—	—	10
D1	—	—	—	—	11
D19	—	—	—	—	12
P143	—	—	—	—	13
?	—	—	F	III/VI	14 (?)
?	—	—	G	IV/XII	15 (?)
?	—	—	L(K?)	— (IX?)	16 (?)

nized (table 6). They include the original Types V, W, X, and Z of Andrewes and Inman and Types 88, 103, and P119 of Boyd. These seven types contain both group-specific and type-specific antigens. The remaining six types (170, P288, P274, D1, D19, and P143) contain type-specific antigens only. There cannot be any doubt that additional types exist and that some of the types described by Boyd have been studied also by other investigators. Boyd's Type 88 is identical with either Type K or Type L of Sartorius and Reploh; clarification on this point is needed. Whether Types F and G of Sartorius and Reploh are identical with any of the types described by Boyd, remains to be seen. The antigenic relationship of the recently described types to other species of the genus *Shigella* and to other genera needs further investigation.

It is evident that the antigenic heterogeneity of the species *S. paradyserteriae* renders a chemical analysis of the antigenic components particularly difficult.

Mention may be made that the differentiation of various antigenic types of the Flexner group is aided by determination of the susceptibility of the strains under investigation to different pure strains of bacteriophage (34).

Dissociation accompanied by changes in the antigenic structure is rather frequently encountered in members of the Flexner group. These variants may differ from the parent colony in the properties of: (a) agglutination; (b) agglutinin-absorption; (c) agglutinogenesis (117, 118). It may be mentioned in passing that, in contradistinction to Shiga's bacillus, members of the Flexner group lack the heterophile sheep-cell hapten, according to Jungeblut and Ross (97). Certain strains of *S. paradyserteriae* share antigens with members of the genus *Salmonella* (Bornstein, Saphra, and Daniels (23)). This latter observation may serve as one of many illustrations of the antigenic interrelationships between different genera and shows that classification of microorganisms should not be based solely on antigenic pattern.

With respect to the pathogenicity of the different types of the Flexner group, it is of interest to note that all of the five Types V, W, X, Y, and Z of Andrewes and Inman may be associated with bacillary dysentery of man (39, 72). Also, some of the new types described by Boyd in 1932 and 1938 (25 to 27) undoubtedly may cause bacillary dysentery, these strains having been isolated (often in almost pure culture) almost exclusively from patients with dysentery. The fact that these patients developed specific agglutinins gives additional evidence of the pathogenicity of the new types of Flexner dysentery bacilli. It is of interest to determine the incidence in other parts of the world of the types of *S. paradyserteriae* described by Boyd.

The question as to whether or not a link exists between the mortality rate and the type of the Flexner group involved deserves further study. Haessler (82) reported a mortality rate of about 30% in his 24 cases of infections due to *S. paradyserteriae*, Type D (cf. table 6), while the mortality rate was considerably lower in infections due to other types. In our series of more than 20 cases of dysentery caused by Type D no death occurred (Neter, unpublished data). Teveli and Tamasi (177) noted no differences in the severity of infections due to different types.

The Widal test for the diagnosis of dysentery due to members of the Flexner group is rendered very difficult because of their antigenic complexity. In the first place, it necessitates the use of several antigenic types. In the second place, no hard and fast rules can be given with respect to agglutinin titers indicative of infection. The titer of normal agglutinins of human sera for members of the Flexner group may range from 1:10 or less up to 1:400 and above, even when identical experimental conditions are observed. Repeated examinations give more reliable results than a single determination of the agglutinin titer. It may be mentioned that sera of patients with infections due to Shiga's bacillus may also agglutinate certain of the Flexner dysentery bacilli. It is regrettable that the Widal test for the diagnosis of infections with

Flexner bacilli has been used quite frequently without due consideration of the points just outlined, and that conclusions regarding the etiology of chronic ulcerative colitis have been based on such results.

S. paradysenteriae is markedly less toxic for experimental animals than *S. dysenteriae*. In contrast to the latter organism, Flexner dysentery bacilli do not produce a powerful exotoxin. On the other hand, living or even killed suspensions of *S. paradysenteriae* may cause lesions and death of animals, attributable to the action of endotoxins present within the bacterial cells. The fact that Flexner dysentery bacilli do not produce an exotoxin may account for the clinical observation that dysentery of man due to these organisms usually appears to be less toxic than dysentery caused by Shiga's bacillus.

8. *S. alkalescens* (Andrewes) Weldin was first described in 1918 by Andrewes (7). It resembles *S. paradysenteriae* in many respects and has been quite frequently confused with it. As a matter of fact, a strain of *S. alkalescens* has been used for the production of a commercially available "Flexner antiserum"! *S. alkalescens* is a non-motile, gram-negative bacillus that grows well on ordinary culture media, including Endo agar. On agar the colonies are usually of the S form; occasionally, R variants may be seen. The biochemical reactions of this organism are very characteristic; the more important ones are summarized in table 4. Like *S. paradysenteriae*, *S. alkalescens* produces acid without gas from glucose, maltose, and mannitol. In litmus milk, following transitory acidification, it produces a characteristic strong alkaline reaction. Indole is always produced. As mentioned above, it differs from Flexner's bacillus in its capacity to produce acid, usually within 24 to 48 hours, from dulcitol, rhamnose, and xylose. Furthermore, it may be differentiated from *S. paradysenteriae* by means of the acid-agglutination test, according to Andrewes (7): *S. alkalescens* is agglutinated in test solutions of pH 2.2 to 3.2, whereas *S. paradysenteriae* is not clumped under identical conditions. These findings, however, have not been completely confirmed by other authors. The possibility has to be considered that the susceptibility to acid-agglutination of S and R forms of Flexner's bacillus may not be the same. From a practical point of view, acid-agglutination is superfluous, because serological methods allow a much more reliable differentiation between these two species.

Antigenically, *S. alkalescens* has been considered until recently to form a homogeneous species (7, 189, 139, 145). Recently, however, Assis (13) has described two different antigenic types (Types I and II). Only a few Type II strains have been described thus far (13). Of more than 40 strains of *S. alkalescens* isolated in this laboratory all were of Type I.

S. alkalescens (Type I) is antigenically related to *S. paradysenteriae*: antisera to *S. paradysenteriae* may agglutinate to high titer *S. alkalescens* and, *vice versa*, antisera to *S. alkalescens* may agglutinate certain types of Flexner dysentery bacilli. However, it has been shown that *S. alkalescens* (Type I) contains a species-specific antigen which is not shared by Flexner dysentery bacilli. Absorption of an antiserum to *S. alkalescens* with a suspension of Flexner dysen-

tery bacilli (Types V, W, X, Y, and Z) eliminates all antibodies to the Flexner bacilli without reducing the titer of agglutinins to *S. alkalescens* (140).

S. alkalescens may be found in the intestinal tract of man free of enteric disease (Snyder (172)). Convincing evidence is accumulating, however, that this organism may cause mild and even severe forms of dysentery or enteritis (12, 13, 64, 133). It also may cause other diseases such as septicemia and infections of the urinary tract (170, 173, 194, 137). Further evidence of its potential pathogenicity is the observation that patients infected with this microorganism may develop specific agglutinins during the course of the disease (139).

9. *S. gallinarum* (Klein) Weldin is a non-motile bacillus. Its more important biochemical characters are shown in table 4. It produces acid from glucose, mannitol, maltose, rhamnose, xylose, and dulcitol, but fails to attack lactose and sucrose. Indole is not formed, and gelatin is not liquefied. It produces H_2S . The reports on its capacity to reduce nitrates and to cause changes of the pH in litmus milk are conflicting (99, 123).

It is interesting to note that a similar organism, *S. gallinarum* (var. *Duisburg*), has been described recently by Müller (131) as the cause of acute gastro-enteritis in man. This microorganism was studied by Kauffmann (98). It differs from *S. gallinarum* in its inability to form H_2S , its slow production of acid from maltose, and its failure to form acid from d-tartrate.

Antigenically, *S. gallinarum*, *S. gallinarum* (var. *Duisburg*), and *Salmonella pullorum* are identical, inasmuch as they contain the O-antigen of Group D of the genus *Salmonella*. It is interesting to point out that this particular somatic antigen is shared also by *E. typhosa*. Thus, species now considered to belong to three different genera contain a common antigen. Some bacteriologists consider *S. gallinarum* as a member of the genus *Salmonella*, more specifically as a variety of *S. pullorum*.

It may be mentioned that *S. gallinarum* differs in its antigenic structure from *S. pfaffii*.

St. John-Brooks and Rhodes (175) have studied *S. gallinarum* in detail and compared it with *S. jeffersonii* of Hadley and his associates. S colonies of the latter were found to be identical with those of *S. gallinarum* in every respect, including antigenic structure. Apparent differences between these two organisms were those of R and S dissociation. Thus, there is no longer any reason to consider *S. jeffersonii* as a distinct species of the genus *Shigella*.

10. *S. pfaffii* (Hadley, *et al.*) Weldin was first isolated from canaries suffering from septicemia. Its distribution and its pathogenicity for animals other than the canary remain to be determined. It is unknown whether this microorganism, like *S. gallinarum*, may cause gastro-enteritis or other diseases in man. In Bergey's Manual of Determinative Bacteriology (19) and in Kelser's Manual of Veterinary Bacteriology (99) it is described as non-motile. According to St. John-Brooks and Rhodes (175), however, it is considered to be motile.

If the latter observation is correct, this organism should be eliminated from the genus *Shigella*.

S. pfaffii produces acid without gas from glucose, mannitol, maltose, rhamnose, and xylose, but not from lactose, sucrose, and dulcitol (table 4). It does not produce indole, does not reduce nitrates, and fails to liquefy gelatin. Its inability to ferment dulcitol and its antigenic structure differentiate it distinctly from *S. gallinarum* (175). A thorough reinvestigation of this species is definitely needed.

11. *S. rettgeri* (Hadley, *et al.*) Weldin was first isolated in 1909 by Rettger from a cholera-like epidemic in chickens. Its distribution and its pathogenicity for various animals and man are not known.

S. rettgeri is a non-motile bacillus;³ it produces acid without gas from glucose, mannitol, and xylose, but does not attack lactose, sucrose, maltose, and dulcitol. Litmus milk is rendered alkaline (table 4). According to Hadley and to Bergey's Manual (19), this organism does not produce indole; St. John-Brooks and Rhodes (175), however, obtained positive results. *S. rettgeri* does not liquefy gelatin. A thorough reinvestigation of this microorganism is desirable, particularly in regard to indole production, incidence, and pathogenic significance.

GROUP III. THE LACTOSE-POSITIVE, MANNITOL-NEGATIVE MEMBERS

Any attempt to discuss the lactose-positive, mannitol-negative members of the genus *Shigella* is confronted with great difficulties. In the first place, organisms of this group (with the exception of *S. gintottensis*) have not been studied adequately and have received but scant attention during recent years. In the second place, the data available in various textbooks, manuals, and dictionaries do not correspond. It appears likely that some of the organisms do not belong to this group or even to the genus *Shigella*. A thorough reinvestigation of the available strains seems necessary. Otherwise, our knowledge of these species will remain as it was several decades ago.

12. *S. gintottensis* (Castellani) Hauduroy, *et al.* was first described by Castellani many years ago. Since then, little has been added to our knowledge of this species. This organism has been recovered from the feces of patients with dysentery and has been considered as the cause of this disease. According to Castellani (37), it is a non-motile, gram-negative bacillus; it produces acid from glucose, and may or may not ferment lactose; it does not attack sucrose,

³ According to Dr. P. R. Edwards, Department of Animal Pathology, University of Kentucky, Lexington, Kentucky (personal communication), a supposed descendant of the original strain of Hadley's culture spreads readily in semi-solid agar when transfers are made from the projecting growth. Continued transfer in this medium gives rise to a very actively motile culture which possesses the cultural and biochemical properties of the original strain. Thus, it appears that *S. rettgeri*, like *S. pfaffii*, should be eliminated from the genus *Shigella*.

mannitol, maltose, and dulcitol; it causes acid and clot formation in litmus milk; it does not form indole (table 7). If certain strains of this species do not produce acid from lactose, as stated by Castellani (37), it is hardly justifiable to include them in this particular group of the genus *Shigella*. Certainly, it should not be classified among the mannitol-fermenting species, as it has been in Bergey's Manual (19).

13. *S. bienstockii* (Schroeter) Bergey, *et al.* is a non-motile, gram-negative bacillus. According to the description in Bergey's Manual of Determinative Bacteriology (1934) (18), it produces acid from glucose and lactose. Ford (66), however, states that it fails to ferment lactose and sucrose and produces

TABLE 7

*Biochemical reactions and antigenic structure of the lactose-positive, mannitol-negative group**
(Data for xylose not available)

	INDOLE	LITMUS MILK	GLUCOSE	LACTOSE	SUCROSE	MANNITOL	MALTOSE	DULCITOL	ANTIGENIC STRUCTURE
<i>Shigella gintottensis</i> (Castellani), Hauduroy, <i>et al.</i> †	—	AC	A	A or —	—	—	—	—	Unknown
<i>Shigella bienstockii</i> (Schroeter), Bergey, <i>et al.</i>	—	AC	A Alk‡	A —†	—†	—			Unknown
<i>Shigella oxygenes</i> (Ford), Bergey, <i>et al.</i>	—	AC	A Alk‡	A —†	—†				Unknown

A = Acid reaction. Alk = Alkaline reaction.

C = Coagulation. — = No indole or acid production.

* Compiled from Bergey's Manual of Determinative Bacteriology (18).

† According to Castellani (36).

‡ According to Ford (66).

an alkaline reaction in glucose broth. It does not form indole. It causes acid and clot formation in litmus milk. Gelatin is not liquefied (table 7).

14. *S. oxygenes* (Ford) Bergey, *et al.* was first isolated by Ford in 1901. It is a non-motile, gram-negative bacillus. According to Ford (66), it does not ferment sucrose and lactose and produces an alkaline reaction in glucose broth. In Bergey's Manual of Determinative Bacteriology (1934) (18), it is described as an organism which produces acid in glucose and lactose. It causes acid and clot formation in litmus milk. It does not form indole and fails to liquefy gelatin.

GROUP IV. THE LACTOSE-POSITIVE, MANNITOL-POSITIVE MEMBERS

15. *S. sonnei* (Levine) Weldin. Late-lactose-fermenting members of the genus *Shigella* were described as early as 1904 by Duval (54, 55) and 1907 by

Kruse (105), and since then by Castellani (36 to 38). However, it was not until 1915, that Sonne (174) proved beyond doubt that such an organism may cause dysentery in man. His conclusions were based on a thorough investigation and his findings may be summed up as follows: (a) Sonne isolated this particular species from patients with bacillary dysentery, in some instances in almost pure culture. (b) These patients developed agglutinins toward this bacillus in titers ranging from 1:25 to 1:250, whereas sera of normal persons very rarely agglutinated this organism in titers of 1:10 or above. (c) With the exception of one carrier, this microorganism was not found in the intestinal canal of normal individuals. (d) Finally, Sonne succeeded in producing a dysentery-like disease in a monkey by feeding one of his strains. Today, this bacillus is referred to as *S. sonnei*, sometimes also as the Duval-bacillus, Duval-Sonne bacillus, Kruse-Sonne bacillus, Kruse-E-bacillus, and *B. ceylonensis* A (Castellani).

The Sonne dysentery bacillus grows well on ordinary media, often somewhat more luxuriantly than either Shiga or Flexner dysentery bacilli. The cultural characters of a large number of strains have been thoroughly investigated by Dienst (48), Koser and Dienst (101), Chinn (40) and Glynn and Starkey (74). Upon isolation on lactose-containing culture media, Sonne's bacillus first appears as a non-lactose-fermenting colony. After incubation for 24 hours on agar the colonies are rather flat, somewhat granular and opaque and have a diameter of 3 to 4 mm. The colonies present a smooth, raised, central zone grading out to a thin slightly irregular edge. Upon further incubation the size of the colony increases. Papillae or daughter colonies appear, which consist of raised, smooth, entire, rounded outgrowths on the surface. Forty-eight hours after their appearance these daughter colonies are usually 1 to 2 mm in diameter. Characteristically, they ferment lactose. A few strains fail to develop papillae even upon incubation for two months. Hall (84) as well as Sears and Schoolnik (165) showed that the late fermentation of lactose is due not to a slow utilization of this carbohydrate, but to the appearance of lactose-fermenting daughter colonies. Essentially the same holds true for the action on sucrose (154). Many different colony forms can be obtained from older cultures on agar. Among them are flat, entire colonies with bevelled edges and a moist shiny surface. According to Glynn and Starkey (74), this particular colony form does not breed true and does not give rise to daughter colonies.

Of great interest are the G or G-like colonies of *S. sonnei*, which were investigated by Dienst (48), Koser and Dienst (101), and Chinn (40). These colonies on agar have a diameter of 0.012 to 0.2 mm as compared to a diameter of 1.5 to 2.5 mm of normal sized colonies. It is worth emphasizing that G or G-like colonies of *S. sonnei* are obtained infrequently and irregularly. Chinn (40) found dwarf colonies to represent only about 1% of all colonies of old strains. Biochemically, the organisms of G colonies are markedly less active than those of the parent colonies. This may be due to (a) the relatively slow growth of the organism and (b) absence or low concentration of certain constitutive enzymes or inability of the organisms to form adaptive enzymes. The

fact that growth-promoting substances such as serum increase the biochemical activity of the G type colony, is in favor of the first possibility. On the other hand, it should be pointed out that even in the presence of serum some G type colonies fail to produce acid from sugars which are fermented by normal sized colonies. Antigenically, certain G type colonies seem to be related to normal strains. Some, but not all G type colonies of *S. sonnei* revert to normal sized colonies upon prolonged incubation on agar or following repeated serial transfers to broth or agar. Thus far, G colonies of *S. sonnei* have not been recovered directly from lesions in man. In this connection it is important to point out that G colonies of *S. equirulis* have been isolated directly from foals. There is no convincing evidence for the existence of filtrable forms of *S. sonnei*. Only one of the strains of Chinn (40) passed through N filters; this strain, however,

TABLE 8

Biochemical reactions and antigenic structure of the lactose-positive, mannitol-positive group

	INDOLE	LITMUS MILK	GLUCOSE	LACTOSE	SUCROSE	MANNITOL	MALTOSE	RHAMNOSE	XYLOSE	DULCITOL	ANTIGENIC STRUCTURE
<i>Shigella sonnei</i> (Levine) Weldin	—	A-AC	A 24 hrs.	A 1-20 days	A 1-20 days	A 24 hrs.	A 24 hrs.	A 24 hrs.	—	—	2 types
<i>Shigella equirulis</i> (de Blicck and van Heelsbergen), Edwards	—	A	A	A	A	A	A	—	A	—	Heterogeneous
<i>Shigella ceylonensis</i> B (Castellani), Weldin	+	AC	A 3 days	A 3-14 days	A 3-7 days	A 3 days	A 3 days	A 3 days	A 3 days	Slight A 3 days or — 14 days	Homogeneous (?)
<i>Shigella madampensis</i> (Castellani), Weldin	+	AC	A 3 days	A 3-14 days	A 3-7 days	A 3 days	A 3 days	A 3 days	A 3 days	—	Probably heterogeneous

A = Acid reaction. + = Indole production.
C = Coagulation. — = No indole or acid production.

did not revert to normal. All other experiments have failed to reveal filtrable forms of this organism.

The biochemical characters of the Sonne dysentery bacillus are summarized in table 8. From a practical diagnostic standpoint it is noteworthy that the fermentation of lactose requires several days and sometimes a few weeks. The differentiation of Sonne's from Flexner's bacillus is aided by testing the capacity to ferment rhamnose: the Sonne bacillus usually produces acid from rhamnose in broth within 24 to 48 hours, whereas most strains of Flexner dysentery bacilli fail to do so or produce acid only after incubation for several days, as shown by Hilgers (92) and confirmed in this laboratory. The inability of the Sonne bacillus to produce indole differentiates this organism distinctly from *S. dispar*, *S. ceylonensis* B and *S. madampensis* (table 8).

Until recently, the Sonne bacillus was considered to represent an antigenically homogeneous species (103, 94). Recent investigations by Glynn and Starkey

(74), however, have revealed two immunological types: Type I contains one of two antigens in predominance, whereas Type II contains both of these antigens in considerable amounts. This conclusion is based upon the following observations: antiserum to Type II agglutinates to full titer both Types I and II strains, whereas antiserum to Type I agglutinates Type II organisms only to a fraction of its titer. Likewise, Type II organisms completely absorb the agglutinins from antisera to both Types I and II, while Type I organisms absorb not more than 50 to 75 per cent of the agglutinins from a Type II serum. These findings are not only of theoretical interest, but also of practical significance. It follows that a serum containing antibodies directed against Type II will agglutinate all agglutinable strains and, therefore, is the serum of choice for diagnostic purposes. In this connection it may be mentioned that some freshly isolated strains of Sonne bacilli are not agglutinated by the homologous antiserum, but may absorb the respective antibodies (69). The absorption experiment, therefore, may be successfully employed when inagglutinable strains are encountered. Attention may be called to the fact that some so-called inagglutinable strains may be agglutinated by applying certain procedures, *e.g.*, prolonged incubation, incubation at higher temperatures (45 C to 55 C instead of 37 C), and by centrifugation of the serum-suspension mixture. Zeithaml and Ecker (196) obtained specific agglutination by centrifugation of Sonne's dysentery bacilli which were otherwise not agglutinated.

The Sonne bacillus shares minor antigenic components with some other members of the genus *Shigella*, namely, *S. paradysenteriae* (74). The cross-reactions between these organisms, however, are not very marked and generally do not interfere with diagnostic agglutination tests.

16. *S. equirulis* (de Blicke and van Heelsbergen) Edwards is the causative agent of a disease of young foals that is characterized by arthritis, nephritis, and septicemia. The organism was most thoroughly studied by Edwards (56). Morphologically, it is highly pleomorphic. It is interesting to note that on agar very young cultures (8 to 10 hours old) frequently show long, filamentous forms and streptococcus-like chains, as well as large yeast-like bodies bearing projections. The microscopic appearance of the organisms of older cultures (14 to 16 hours) parallels to a certain extent the characters of the colony. Rough, mucoid colonies consist almost entirely of short oval rods, whereas smooth colonies contain many long filamentous forms and streptococcus-like chains. Edwards made the important observation that rough colonies of *S. equirulis* are always mucoid and that non-mucoid colonies are always smooth. The trend of the variation in artificial cultures is from rough to smooth. Smooth mucoid colonies are found as a transitional stage.

R forms of *S. equirulis* are very mucoid; they have a dry, dull surface; the edges are undulate and the surface is extremely rough. In broth growth appears first on the side of the culture tube; later, a light pellicle is formed on the surface, and the sides of the tube are covered with masses of bacteria. Still later, a diffuse growth may occur. Smooth colonies on agar, on the other hand,

are flat or slightly raised, non-mucoid, and have a perfectly smooth surface; in broth an even clouding occurs. It must be noted, however, that the relation of the features of cell morphology and mucoid nature of the rough phase, as reported by Edwards, differs from findings in other species and that such an expert on problems of bacterial dissociation as Hadley (79) raises the question as to whether the mucoid-rough cultures of Edwards may have been impure. Nevertheless, the observation of Edwards of the existence of a mucoid phase in a member of the genus *Shigella* itself is of great importance.

In addition to rough and smooth colonies of *S. equirulis*, dwarf colony variants may occur. These dwarf colonies are composed of short oval rods. On agar they appear after an incubation period of 4 days or longer and usually have the same characters in regard to rough or smooth consistency as the parent colonies (56). In contrast to normal strains, which grow readily in extract broth, these dwarf variants do not grow at all or only very poorly in this culture medium.

Biochemically, *S. equirulis* forms acid from glucose, levulose, galactose, maltose, lactose, sucrose, xylose, raffinose, and mannitol; no acid is produced from rhamnose, dulcitol, and sorbitol. Indole is not formed and gelatin is not liquefied. It reduces nitrates to nitrites. Litmus milk is rendered acid and some strains cause coagulation of the milk and reduction of the litmus. As may be seen from table 8, *S. equirulis* may be readily distinguished from the Sonne dysentery bacillus by its inability to ferment rhamnose and its capacity to ferment xylose.

Antigenically, *S. equirulis* is a heterogeneous species. Both smooth and rough colonies of a single strain possess a common antigen. It is interesting to note that, as shown by Edwards (56), dwarf colonies are antigenically related to the normal strains.

Edwards (56) has made an observation of great general interest, namely, that both rough and smooth forms as well as dwarf colonies of *S. equirulis* can be isolated from the tissues of infected foals, indicating that all three forms of this microorganism may be pathogenic.

It has been suggested that this species might be a member of the genus *Actinobacillus* (unpublished suggestion; cf. Bergey's Manual (19)).

17. *S. ceylonensis* B (Castellani) Weldin. 18. *S. madampensis* (Castellani) Weldin. *S. ceylonensis* B and *S. madampensis* were originally described by Castellani in 1907 and 1911, respectively; the former microorganism was isolated from the feces of patients with the clinical signs of dysentery, and the latter from patients with colitis and cystitis. In 1918, Andrewes (7) described certain lactose-fermenting microorganisms under the name of *Bacillus dispar*. Subsequent investigations revealed that strains described as *Bacillus dispar* are identical with either *S. ceylonensis* B or *S. madampensis*. Thus, at the present time, *S. dispar* can not be recognized as a distinct species. *S. ceylonensis* B and *S. madampensis* show only minor differences. It seems reasonable to suggest, therefore, that the organisms under consideration be classified as a

single species (*S. castellanii*), just as *S. paradysenteriae* comprises organisms with certain biochemical and antigenic differences.

Colonies on agar of strains classified as *S. dispar* appear very similar to colonies of the Sonne bacillus. They, too, may show secondary papillae. The biochemical characters of *S. ceylonensis B* and *S. madampensis* are almost identical, as may be seen from table 8. *S. ceylonensis B* differs from *S. madampensis* in its ability to produce acid from dulcitol. Indole production distinctly differentiates *S. ceylonensis B* and *S. madampensis* from the Sonne bacillus. Furthermore, according to Forsyth (68), strains identified as *S. dispar* are methyl-red-positive, whereas *S. sonnei* is not.

The antigenic structure of these organisms is not as yet fully elucidated. Castellani (36 to 38) reported that both *S. madampensis* and *S. ceylonensis B* are antigenically homogeneous species. However, it should be noted that according to Glynn and Starkey (74) strains labeled as *S. dispar*, which were identical with strains of *S. madampensis*, proved to be antigenically heterogeneous. Furthermore, Forsyth (68) found that strains identified as *S. dispar* form an antigenically heterogeneous species. Further studies are needed before final conclusions can be drawn in regard to the antigenic pattern of the species under consideration. Then, it will be possible also to determine the antigenic relationship of these organisms to other species of the genus. Minor antigenic relationships between *S. dispar* and Sonne and Flexner dysentery bacilli were found by Watanabe (186) and by Welch and Mickle (188).

No conclusive evidence is available at the present time that these organisms are a cause of epidemic or endemic dysentery of man. Members of this group of organisms may be found in the feces of healthy individuals and of patients with or without intestinal diseases. Johnston and Kaake (95) reported cases of enteritis in children from whom *S. dispar* was isolated. It remains to be seen whether these organisms are the primary incitants of the disease or only secondary invaders. Occasionally, these organisms may cause infections of the urinary tract and purulent lesions originating from the intestinal canal.

GENERAL CONSIDERATIONS

Many species of the genus *Shigella* have been studied thoroughly in the past and much information of general bacteriological and immunological interest has been brought to light. However, it is evident from a review of the available reports that other species, now classified as members of this genus, have not been adequately investigated, and little is known in regard to their cultural characters, biochemical activities, and antigenic structure. It is safe to assume that some of these microorganisms may not even belong to the genus. It seems highly desirable that competent investigators collect and study as many strains as possible of these little known species such as *S. septicaemiae*, *S. minutissima*, *S. pfaffi*, *S. rettgeri*, and others. Only then, will it be possible to reconsider their taxonomic position, to identify with accuracy strains isolated from various sources, and to determine their significance in diseases of man and animals.

Until recently, our knowledge of the specific growth requirements of the members of the genus has been inadequate. Investigations similar to those of Koser and his associates, when they are extended to include all the members of the genus, may yield interesting information and aid greatly in the classification of these microorganisms.

One of the most significant recent contributions to our knowledge of the genus is that of Boyd. His demonstration of a group-specific antigen in certain types of *S. paradysenteriae* may form a basis for similar investigations of other species and genera. The observations of Boyd are also of practical importance and should be considered in any attempt to produce either active or passive immunity to infections with the Flexner group of dysentery bacilli. The chemical analysis of antigenic components of some species of the genus has yielded interesting information during the last few years. Such investigations have been particularly fruitful at the hands of Morgan and his associates. It is to be hoped that their studies on Shiga's dysentery bacillus will be extended to other species.

One of the least understood problems connected with the genus *Shigella* in particular is that of pathogenicity. At the present time, with the exception of the Shiga exotoxin, very little is known in regard to the factors in dysentery bacilli responsible for dysentery of man, and it has not been explained why one species may cause epidemic outbreaks of this disease and a very closely related species does not. Future investigations may shed light on these problems.

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